THE PRELIMINARY SCREENING OF THE ENDOPHYTIC FUNGAL EXTRACTS OF *Acalypha wilkesiana* FOR ANTIMICROBIAL ACTIVITY

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Abstract: Endophytic fungi have been shown to be a promising source of novel antimicrobial moieties. This study was carried out to determine the antimicrobial profile of the endophytic fungal isolates from *Acalypha wilkesiana*. The leaves of *A. wilkesiana* was washed in running water, sterilized and then inoculated onto freshly prepared Sabouraud dextrose agar (SDA). The pure isolates were then subjected to solid state fermentation using rice medium. This was followed by the extraction of the product of fermentation using Ethyl acetate and the extracts were concentrated using rotary evaporator set at 50^0C. The antimicrobial activity of the extracts was then evaluated against the clinical isolates: *Salmonella typhi*, *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtillis*, *Candida albicans* and *Aspergillus fumigatus*. A total of five (5) endophytic fungal isolates were obtained from the leaf sample, identified by their unique colony morphology and were labeled M1, M2, M3, L2 and L3. The endophytic fungal extracts showed varying degree of antimicrobial activities against the clinical isolates with all the extracts showing more potent antimicrobial activities against the clinical isolates than the known antibiotics at the concentration of 1 mg/mL. The results of this study suggest that *A. wilkesiana* have antimicrobial activity and further studies should be carried out to identify the endophyes to species level as well as the bioactive compounds responsible for this activity it elicits.

Keywords: *Acalypha wilkesiana*, antimicrobial activity**,** endophytic fungi, extracts, preliminary

Introduction

Endophytic fungi otherwise known as endophytes reside naturally in the tissues of plant parts such as leaves, roots, stems, petioles, etc., without causing any obvious disease presentations (Petrini, 1991). The relationship that exists between the endophytes and the host plants is mutualistic, where they receive protection and nutrition from the host plant and the hostplant in turn benefit from this relationship through enhanced ability to survive in any environment and increased resistance to pathogens, herbivores and various abiotic stresses tolerance (Yang *et al*., 1994; Clay *et al*., 1986). The fungi and bacterial seem to be the most common microbes existing as endophytes, but the most frequently isolated are the endophytic fungi (Staniek *et al*., 2008). The discoveries of endophytes residing in unique ecological niches piqued the interest of scientists into further research leading to the isolation of more endophytes and their natural products (Akpotu *et al*., 2017a; Arnold *et al*., 2011; Yu *et al*., 2010). It has been observed from researches that medicinal plants with great use in ethno- medicine harbours endophytes with great metabolic diversities with their attendant capacity to produce bioactive compounds, which could then be developed into agents used in therapy for antifungal, antibacterial and even antiviral purposes (Singh *et al*., 2021; Han *et al*., 2013; Kharwar *et al*., 2011; Strobel *et al*., 2003).

The use of medicinal plants for treatment of common ailments traditionally in Nigeria and indeed most parts of sub-Saharan Africa dates back several centuries ago (Sofowora, 1983). *Acalypha wilkesiana* (Euphorbiaceae) Muell Arg, a known medicinal plant, is commonly used in most African countries and Asia (Mothana *et al*., 2008; Sofowora, 1993). It is commonly called red acalypha and has been known to be used for the treatment of some form of skin infections in children by bathing them with the cold extracts of the leaves (Adesina *et al*., 2000). In Trinidad, it has found use for the treatment of body swellings as well as cold (Gills, 1992). Some other researchers also found out that the plant has been used in ethno medicine for the treatment of malaria as well as some gastrointestinal tract complaint (Akinyemi *et al*., 2006; Akinde, 1986).It is believed that the biological activities elicited by this plant and indeed all medicinal plants used in ethno medicine could be due to certain bioactive compounds they contain (Akpotu *et al*., 2017b).

This research, therefore seek to evaluate the antimicrobial activity of the endophytic fungal extracts of *A. wilkesiana* from a difficult terrain in Sagamu, Nigeria.

Materials and Methods

Plant sampling and study area

Apparently healthy leaves of *Acalypha wilkesiana* (Euphorbiaceae) Mull.Arg were collected from swampy areas on the Sagamu Campus of Olabisi Onabanjo University, Ogun State, Nigeria.

Isolation of endophytic fungi

The apparently healthy leaves of *A. wilkesiana* so harvested were thoroughly washed in running water to remove all form of debris. The leaves were then surface sterilized to eliminate epiphytes by immersion in 100% ethanol for 1 min, 2% Sodium hypochlorite for 30 seconds followed by rinsing in sterile distilled water for 5 minutes and blotted. The lamina and midribs of the leaves were cut with a sterile surgical blade into small pieces (1 – 1.5 cm) and inoculated onto four petri dishes containing Sabouraud dextrose agar (SDA) medium supplemented with chloramphenicol (250 mg/100mL) to inhibit the growth of bacterial contaminants.The plates were sealed with paper tapes and then incubated at room temperature (25 – 27^0C). This was followed by sub-culturing to obtain pure cultures.

Fermentation and extraction of the metabolites

For the solid-state fermentation, the pure cultures were inoculated onto rice medium in Erlenmeyer flask prepared according to Akpotu *et al*. (2017a). They were then allowed to stand for 28 days undisturbed. The secondary metabolites were then extracted with 500 mL ethyl acetate, filtered to obtain the filtrate using suitable filter paper (Whatman No. 1) and finally the extracts were concentrated with rotary evaporator set at 50^0C.

Antimicrobial evaluation

This was done using the procedure of Akpotu *et al*. (2017b) with a little modification. The antimicrobial activity of the fungal extracts was challenged against some clinical bacterial isolates such as *Salmonella typhi*, *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtillis* and some clinical

fungal isolates such as *Aspergillus niger* and *Candida albicans*. A concentration of 1 mg/mL was prepared from each of the fungal extracts using dimethyl sulphoxide (DMSO). Exactly 20 mL of molten Mueller-Hinton agar (for bacterial isolates) and 20 mL of molten SDA (for fungal isolates) were dispensed into 90 mm petri dishes and allowed to solidify. This was followed by the swabbing of 0.5 McFarland's standard of the overnight culture of the test isolates aseptically on the agar plates. Then, five (5) holes were dug out in each plate by means of a 6 mm cork borer, to which 20 µL of each of the extract in varying concentrations were dispensed into and the positive control (gentamicin for bacterial isolates and fluconazole for fungal isolates) dispensed in the hole at the center. The DMSO was used as negative control. All the plates were then allowed to stand for 1 h to allow for prediffusion and then subsequently incubated at 37^0C for 24 h (bacterial isolates) and at 27^0C for 48 h (fungal isolates) respectively. The inhibition zone diameters (IZDs) were measured and recorded. The above procedure was done in triplicates and the mean IZDs recorded.

Statistical analysis

The results were analyzed using the Statistical Package for Social Sciences (SPSS) version 22.0 by one way analysis of variance (ANOVA) and significance taken at $p<0.05$.

Results and Discussion

From Table 1, we could observe that five (5) endophytic fungi were isolated from the leaves (midrib and lamina) of *A. wilkesiana*. This shows the possibility of isolating endophytic fungi from medicinal plants, especially those growing in an unusual ecological niche such as swampy environment in our own case. This is therefore in accordance with earlier researches (Singh *et al*., 2021; Akpotu *et al*., 2017b; Hussain *et al*., 2014; Han *et al*., 2013; Hata and Stone, 2008; Strobel and Daisy, 2003; Strobel, 2003).

Table 1: The endophytic fungi isolated from the leaf of *Acalypha wilkesiana*

Isolate Source	
Lamina	**Midrib**
L2	M1
L3	M2
	M3

The symbiotic relationship that exists between the endophytes and their host plants such that host plant enjoys long life span because of the stamina and immunity the endophytes confer on the host plant and the host in turn provides shelter and nutrients for the endophytes, would also ensure the continuous isolation of endophytes from these medicinal plants (Strobel and Daisy, 2003). This may also be responsible for the endophytic fungi isolated from this plant. It has been said that the incidence of endophytes infestation of host plants is affected by the variables of altitude, humidity as well as the density of the host canopy (Petrini and Carroll, 1981). These variables may have affected the number of endophytic fungi so isolated.

Table 2: The inhibitory zone diameter of M1 extract against the test organisms

Test Organisms	Inhibition zone diameter (mm) M1 Extract Concentration (mg/mL)						
	1	**0.5**	**0.25**	**0.125**	**0.0625**	**Gentamicin (10 µg/mL)**	**DMSO**
ST	22.3	20.4	19.5	18.3	17.5	20.0	0.0
EC	22.7	21.0	19.7	18.5	17.5	20.0	0.0
SA	22.7	21.7	20.5	19.6	18.0	20.0	0.0
BS	21.0	20.5	19.6	18.5	17.0	20.0	0.0
Fluconazole	**DMSO (10µg/mL)**						
CA	20	19	18	17	17	18.0	0.0
AN	19	16	16	15	15	18.0	0.0

ST: *Salmonella typhi*, **EC**: *Escherichia coli*, **BS**: *Bacillus subtillis*, **SA:** *Staphylococcus aureus*, **CA**: *Candida albicans*, **AN**: *Aspergillus niger*, **DMSO**: Dimethyl sulfoxide.

Table 3: The inhibitory zone diameter of M2 extract against the test organisms

Test organisms	Inhibition zone diameter (mm) M2 Extract Concentration (mg/mL)						
	1	**0.5**	**0.25**	**0.125**	**0.0625**	**Gentamicin (10µg/mL)**	**DMSO**
ST	21.0	18.0	18.0	16.0	15.0	20.0	0.0
EC	20.5	19.5	18.2	17.0	17.0	20.0	0.0
SA	22.0	21.0	19.0	18.0	17.0	20.0	0.0
BS	25.0	22.0	20.5	17.0	17.0	20.0	0.0
	Fluconazole	**DMSO (10µg/mL)**					
CA	25.0	23.0	21.0	18.0	17.0	18.0	0.0
AN	24.0	24.0	20.0	19.0	16.0	18.0	0.0

ST: *Salmonella typhi*, **EC**: *Escherichia coli*, **BS**: *Bacillus subtillis*, **SA:** *Staphylococcus aureus*, **CA**: *Candida albicans*, **AN**: *Aspergillus niger*, **DMSO**: Dimethyl sulfoxide.

Table 4: The inhibitory zone diameter of M3 extract against the test organisms

Test Organisms	Inhibition zone diameter (mm) M3 extract Concentration (mg/mL)						
	1	0.5	0.25	0.125	0.0625	Gentamicin (10µg/mL)	DMSO
ST	22.0	18.0	17.0	16.5	16.0	20.0	0.0
EC	21.0	20.5	18.0	18.0	16.0	20.0	0.0
SA	25.0	21.0	19.0	18.0	17.0	20.0	0.0
BS	21.0	18.0	17.0	16.0	15.0	20.0	0.0
Fluconazole	**DMSO (10 µg/mL)**						
CA	24.0	22.0	19.5	19.0	18.0	18.0	0.0
AN	21.0	19.0	18.0	17.5	17.0	18.0	0.0

ST: *Salmonella typhi*, **EC**: *Escherichia coli*, **BS**: *Bacillus subtillis*, **SA:** *Staphylococcus aureus*, **CA**: *Candida albicans*, **AN**: *Aspergillus niger*, **DMSO**: Dimethyl sulfoxide.

Table 5: The inhibitory zone diameter of L2 extract against the test organisms

Test Organisms	Inhibition zone diameter L2 extract Concentration (mg/mL)						
	1	0.5	0.25	0.125	0.0625	Gentamicin (10µg/mL)	DMSO
ST	20.4	19.5	17.5	16.0	16.0	20.0	0.0
EC	20.5	19.0	18.0	16.0	15.0	20.0	0.0
SA	23.0	21.0	20.5	19.0	18.0	20.0	0.0
BS	22.0	21.0	19.0	18.0	17.0	20.0	0.0
Fluconazole	**DMSO (10 µg/mL)**						
CA	25.0	22.0	21.0	19.0	18.0	18.0	0.0
AN	19.0	16.0	16.0	15.0	14.0	18.0	0.0

ST: *Salmonella typhi*, **EC**: *Escherichia coli*, **BS**: *Bacillus subtillis*, **SA:** *Staphylococcus aureus*, **CA**: *Candida albicans*, **AN**: *Aspergillus niger*, **DMSO**: Dimethyl sulfoxide.

Table 6:The inhibitory zone diameter of L3 extract against the test organisms

Test Organisms	Inhibition zone diameter L3 extract Concentration (mg/mL)						
	1	0.5	0.25	0.125	0.0625	Gentamicin (10 µg/mL)	DMSO
ST	20.5	19.0	18.0	16.0	15.0	20.0	0.0
EC	21.0	18.3	17.5	17.0	16.0	20.0	0.0
SA	22.0	20.5	19.0	18.0	17.0	20.0	0.0
BS	21.0	20.5	19.0	17.0	15.0	20.0	0.0
Fluconazole	**DMSO (10 µg/mL)**						
CA	19.0	17.0	16.0	15.0	14.0	18.0	0.0
AN	18.5	17.0	16.0	15.0	15.0	18.0	0.0

ST: *Salmonella typhi*, **EC**: *Escherichia coli*, **BS**: *Bacillus subtillis*, **SA:** *Staphylococcus aureus*, **CA**: *Candida albicans*, **AN**: *Aspergillus niger*, **DMSO**: *Dimethyl sulfoxide.*

Tables 2 – 6 show the antimicrobial susceptibility profile of the different endophytic fungal extracts at various concentrations against the six (6) different clinical isolates and comparing their performances with that of the known antibiotics. At a concentration of 1 mg/mL, all the endophytic fungal extracts showed more potent antimicrobial activities than the known antibiotics. This could be because the extracts are still in their crude form, containing other molecules in addition to the one of interest. It could also be observed that the potency of the fungal extracts decreases down the concentration gradients. This corroborates earlier work (Akpotu *et al*., 2017b; Hussain *et al*., 2014; Selim *et al*., 2011). On the whole, 46.7% of the M2 fungal extracts at varying concentrations showed more potent antimicrobial activities than the known antibiotic (Table 3), whereas, for the L3 extracts 26.7% showed more potent antimicrobial activities than the standard. This simply shows that the M2 extract is a more potent than the L3 extracts in terms of their antimicrobial activities.

Conclusion

The outcome of this study shows that endophytic fungal extracts from *A. wilkesiana* could be a source of novel antimicrobial agent, since it shows greater potency compared to the standard at certain concentration. However, further studies have to be carried out to determine the exact bioactive compounds responsible for this antimicrobial activity and possibly formulate it into suitable dosage form to further enhance our repertoire of antimicrobial agents available for therapy for the benefit of mankind as well as livestock.

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Conflict of Interest

We wish to state that we do not have any conflict of interest as far as this research is concerned.

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